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(54) Diagnosis and grading of cervical intraepithelial neoplasia (CIN).

(57) A method of grading cervical intraepithelial neoplasia (CIN) by treating tissue from the cervix with neuraminidase, staining the treated tissue using immunohistochemical techniques with a monoclonal antibody to the CD15 antigen (3–fucosyl–N–acetyllactosamine), determining the extent of staining in normal tissue compared to that of dysplastic tissue and classifying the extent of staining to provide an indication of the grade of CIN present.

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Diagnosis and grading of Cervical Intraepithelial Neoplasia (CIN)

This invention relates to the diagnosis and grading of cervical intraepithelial neoplasia.

In addition to the changes of overt cancer in the cervix changes are recognised in the cervical mucosa which are regarded as precursors of cancer. These changes are called dysplastic changes and are present in grades of severity in the disease spectrum entitled cervical intraepithelial neoplasia (CIN). The detection and treatment of the changes of CIN form the basis of cervical screening in an attempt to prevent the development of cervical cancer. In this screening program a cervical smear is first taken to give indication whether further investigation is necessary. In the event of positive result a biopsy is taken and the tissue obtained is stained and assessed by a pathologist.

The routine histopathological grading of CIN, carried out on sections stained with haemotoxylin and eosin (H&E) is known to be subjective, open to great intra- and interobserver error. The grading of CIN is not important as regards patient treatment, but may influence patient follow-up, and

more consistent grading is desirable for descriptive and epidemiological purposes.

According to the present invention there is provided a method of grading cervical intraepithelial neoplasia (CIN) by treating tissue from the cervix with neuraminidase, staining the treated tissue using immunohistochemical techniques with a monoclonal antibody to the CD15 antigen (3-fucosyl-N-acetyllactosamine), determining the extent of staining in normal tissue compared to that of dysplastic tissue and classifying the extent of staining to provide an indication of the grade of CIN present.

The monoclonal antibody is preferably MC2, produced at Ninewells Hospital, Dundee, which is a mouse IgM monoclonal antibody, and it is used in the form of tissue culture supernatant or ascitic fluid at a dilution of, for example 1:1000. Antibody MC2 is produced by the standard method of Kohler & Milstein by fusion of the NS1 myeloma line with spleen cells from a mouse immunised three times intraperitoneally and intradermally with human granulocytes. The antibody is one of many with similar specificity raised in this and other laboratories. All of these antibodies recognise the CD15 or 3-fucosyl-N-acetyllactosamine antigen found originally on human granulocytes and a variety of other human cell types. Similar antibodies are available commercially, such as Leu-M1 (Becton-Dickinson Ltd.; Dako M1, Dako Ltd.; MC1, Seralab Ltd.). All of the antibodies give the same staining pattern on cervix although our own results show MC2 to give stronger staining. The production and characterisation of the antibody has been described in J. Clin. Pathol. (1985) 38:521-529 (McCarthy, N.C., Simpson, J.R.M., Coghill, G. & Kerr, M.A.).

It has previously been shown by Howie & Brown (J. Clin.

Pathol. (1985) 38:409-416) that treating cervical tissue with the enzyme neuraminidase prior to staining with their own monoclonal antibody to the CD15 antigen results in enhanced staining of endocervical glands, mucus, and cells in the full thickness of normal stratified squamous epithelium. The inventors have surprisingly found that contrary to the finding of Howie & Brown the present invention produces a clear differentiation between a zone of stained supra-basal cells and a zone of unstained basal cells in normal squamous epithelium, and that the present method thereby can provide specific information on the degree of cellular differentiation in cervical squamous epithelia in health and disease.

The CD15 antigen (3-fucosyl N-acetyllactosamine) is present on the outer cell membrane of cervical squamous epithelial cells. Staining sections of cervical biopsies with MC2 clearly demonstrates the zone of supra-basal differentiated cells in the normal squamous epithelium. Staining with MC2 also demonstrates the diminished proportion of this zone occurring with grades of CIN, reflecting progressive de-differentiation of the epithelium. In immature squamous metaplastic epithelium absence of cytoplasmic differentiation is reflected by lack of staining.

As expression of the antigen by cervical squamous cells mirrors cytoplasmic maturity and is a marker of cellular differentiation, staining colposcopic biopsies with MC2 may aid the routine histopathological grading of CIN.

Embodiments of the present invention will now be described by way of illustration in the following Example.

EXAMPLE

MC2, a mouse IgM monoclonal antibody, was used in the form

of ascitic fluid at a dilution of 1:1000 throughout this Example.

H&E sections from colposcopic biopsies previously received by the routine histopathology department were reviewed and those biopsies considered to show the histological changes typical of a range of squamous epithelial lesions were selected. The biopsy material had been fixed in 10% buffered formalin and paraffin processed. Sections were pre-treated with neuraminidase (Sigma Chemical Co. Ltd., Poole; Clostridium Perfringens) at a dilution of 1 unit/ml for 15 hrs at 37°C. Staining was completed with an indirect immunoperoxidase technique making use of biotinylated anti-mouse IgM as part of an avidin biotin complex (ABC) Vectastain kit (Vector Laboratories, Peterborough).

The number of biopsies examined together with the histological diagnosis and pattern of staining with MC2 are shown in Table 1.

In areas of normal cervical squamous epithelium MC2 stained antigen on the cell membrane of supra-basal cells showing evidence of cytoplasmic maturity, and clearly demonstrated a broad zone of these cells representing around 3/4 of the total epithelial thickness. Staining of epithelium containing koilocytotic cells demonstrated a similar proportion of stained supra-basal cells; supra-basal virus-infected cells retained antigen expression. Cells of immature squamous metaplasic epithelium did not stain, and demonstrated few cytological features of cytoplasmic maturation. There was staining of mucus within overlying endocervical cells. Examples of sections stained with MC2 from material considered to show the range of grades of CIN clearly demonstrated changes in the relative proportion of

the zone of stained cells against the total epithelial thickness. In lesions of CIN I there was a decreased proportion of supra-basal stained cells. Cells containing abnormal nuclei were seen in the full epithelial thickness but some superficial cells did show evidence of cytoplasmic maturity and these cells expressed the antigen. In CIN II the epithelium contained a larger proportion of undifferentiated cells and fewer cells stained for the antigen. There was often no staining of cells in CIN III; alternatively only the most superficial of cells expressed antigen.

CIN is a disease spectrum characterised by an epithelium showing disturbance of cellular maturation and organisation and in which cells display nuclear and cytoplasmic abnormalities consistent with malignant cells. Changes occurring in cells at the lower end of the disease spectrum (CIN I) are qualitatively similar to those at the upper end (CIN III). However, the observed changes do vary quantitatively depending on the degree of epithelial differentiation. In CIN I and CIN II cells at all levels of the epithelium contain nuclei with the features associated with malignancy, but cells of the upper and middle strata demonstrate cytoplasmic differentiation. With increasing degrees of atypia the cells progressively lose cytoplasmic maturation and eventually the whole epithelium is replaced by undifferentiated cells.

In this Example it is demonstrated that in normal cervical squamous epithelium the antigen is expressed on the cell membrane of a broad zone of supra-basal cells showing histological evidence of cytoplasmic differentiation. When the squamous epithelium comprises cells which lack cytoplasmic maturity, such as in immature squamous metaplastic epithelium, there is corresponding absence of

expression of the antigen. In CIN the antigen is expressed only on mature superficial cells including cells showing cytoplasmic maturity but containing atypical nuclei. With increasing degrees of atypia fewer cells achieve cytoplasmic maturity and this de-differentiation of the epithelium is mirrored by a progressive loss of cells expressing the antigen. Thus expression of the antigen reflects cytoplasmic maturity and is a marker of cellular differentiation in cervical squamous epithelial cells. Staining colposcopic biopsies for the antigen with MC2 clearly demonstrates the proportion of differentiated cells in cervical squamous epithelium and may provide a diagnostic aid in the histopathological grading of CIN. The limitations of assessing dysplastic changes on differentiation alone are highlighted by two instances; the lack of cellular maturity in metaplastic epithelium renders assessment of differentiation largely valueless as an aid to grading CIN arising in this type of epithelium. In addition inflammatory lesions result in an increased proportion of immature basaloid mucosal cells; differentiation must therefore always be assessed in conjunction with the other cytological parameters of neoplasia for the accurate grading of CIN.

Two methodological factors proved to be of importance in this study. Neuraminidase treatment of sections enhances staining of the antigen in some tissues by uncovering additional residues. In our experience in the cervix this results in much clearer staining of antigen on the cell membrane without altering the number or distribution of cells expressing the antigen. Staining is also clearer using specific anti-mouse IgM second antibodies rather than anti-mouse IgG (H&L chain reagents) to develop the sections. The presence of the antigen in neutrophil polymorphs and endocervical mucins provides an inherent positive staining

control in most sections and when these are not strongly positive staining should be repeated. Of particular interest are occasions where CIN occurs concurrently with koilocytosis, a frequent diagnostic problem. Groups of koilocytotic cells retain the antigen so they can be stained even when surrounded by poorly maturing epithelium. This allows discrimination between dysplastic and koilocytotic cells, of great advantage when assessing the grade of CIN in these instances.

It is known that the lack of cellular differentiation shown by cervical neoplastic cells results in loss of cellular cohesion, linked to a decrease in cellular desmosomes and cell junctions, an absence of surface microridges, and development of surface microvilli. The antigen is known to be expressed on several glycoproteins found on the surface and in the intra-cellular granules of neutrophils. Among these glycoproteins are the members of the LFA (lymphocyte function associated) family of molecules which are found on all leucocytes and are involved in mediating cellular adhesion. The LFA family of proteins is related to a much larger super-family which includes the fibronectin, laminin and vitronectin receptors; proteins which are involved in interaction with the extra-cellular matrix, controlling cell migration and localisation during morphogenesis. In the cervix the antigen is part of the cell glycocalyx in supra-basal squamous mucosal cells and may also have a role in cellular adhesion, helping to maintain the integrity of the epithelium. We have demonstrated loss of the antigen in CIN and this may contribute to the loss of cellular cohesion.

Modifications and improvements may be made without departing from the scope of the invention.

TABLE 1

pattern of expression of the CD15 antigen in cervical squamous epithelia stained with MC2

Type (No. of sections examined)	Pattern of expression
Normal (15/15)	Strong staining of broad zone of suprabasal cells around 3/4 of the total mucosal thickness.
Immature Metaplasia (6/6)	No cellular staining.
Mature Metaplasia (6/6)	Staining of narrow zone of superficial cells.
Koilocytosis (6/6)	Strong staining of broad zone of suprabasal cells. Suprabasal virus infected cells also stain.
CIN I (12/12)	Reduction in zone of suprabasal stained cells. Cells with nuclear atypia but with some cytoplasmic differentiation stain.
CIN II (12/12)	Further reduction in zone of suprabasal stained cells.
CIN III (12/12)	Either only most superficial of cells stain or no cellular staining.

CLAIMS

1. A method of grading cervical intraepithelial neoplasia (CIN) by treating tissue from the cervix with neuraminidase, staining the treated tissue using immunohistochemical techniques with a monoclonal antibody to the CD15 antigen (3-fucosyl-N-acetyllactosamine), determining the extent of staining in normal tissue compared to that of dysplastic tissue and classifying the extent of staining to provide an indication of the grade of CIN present.

2. A method as claimed in Cláim 1, wherein the monoclonal antibody is MC2.

3. A method as claimed in Claim 1 or 2, wherein the monoclonal antibody is in solution in ascitic fluid.

4. A method as claimed in Claim 1, 2 or 3, wherein the tissue is treated with neuraminidase at a dilution of 1 unit per ml at 37^{o}C.

5. A method as claimed in any one of the preceding Claims, wherein the staining is completed by an indirect immunoperoxidase technique.